Amendments to the Claims

42. A multi-gene expression profile of a sample comprising a collection of linearly amplified specific nucleic acid messages, wherein said amplified specific nucleic acid messages each have an abundance which reflects the relative representation of specific nucleic acid messages within the sample.
43. The multi-gene expression profile of claim 42, wherein said amplified specific nucleic acid messages have been amplified simultaneously with RNA polymerase and primer linked to RNA polymerase promoter.
44. The multi-gene expression profile of claim 42, wherein said amplified specific nucleic acid messages comprise aRNA.
45. The multi-gene expression profile of claim 42, wherein said amplified specific nucleic acid messages comprise cDNA.
46. The multi-gene expression profile of claim 42, wherein said amplified specific nucleic acid messages are hybridized to a hybridizing target.
47. The multi-gene expression profile of claim 46, wherein said amplified specific nucleic acid messages are hybridized to a hybridizing target by northern or Southern blot.
48. The multi-gene expression profile of claim 42, wherein said sample is a mammalian cell.
49. The multi-gene expression profile of claim 48, wherein said sample is a cell from brain, spleen, bone, heart, vascular tissue, lung, kidney, liver pituitary, endocrine gland, lymph node, or tumor.
50. The multi-gene expression profile of claim 48, wherein said sample is a blood cell.
51. The multi-gene expression profile of claim 48, wherein said sample is a neural cell.
52. The multi-gene expression profile of claim 48, wherein said sample is a single cell.
53. A multi-gene expression profile of a sample comprising a collection of linearly amplified specific nucleic acid messages, wherein said amplified specific nucleic acid messages have been amplified simultaneously with RNA polymerase and primer linked to RNA polymerase promoter.
54. The multi-gene expression profile of claim 53, wherein said amplified specific nucleic acid messages are hybridized to a hybridizing target.

R marks/Arguments

Summary Of Th Inv ntion

The amplification of heterogeneous populations of messenger ribonucleic acid (mRNA) is quite beneficial to researchers who wish to view the complexity of mRNA in relative representation to the population of mRNA. Analysis of gene expression via research of the mRNA had been hindered by the high complexity of the mRNA, the relatively low abundance of many important expressed messages, the spatially limited expression of these messages, until this invention by Van Gelder et al.

As stated in RNA Interviews, "The RNA amplification method you [Dr. James Eberwine, one of the inventors] developed is now used all over the world for preparing RNA samples for gene array analysis." (http://ambion.com/techlib/tn/94/9421.html) Based on the fact that mRNA sequences have a poly A end, Van Gelder et al. devised a method to produce a multi-gene profile in which a collection of different nucleic acids therein are in linear proportion to the mRNA species in the sample and therefore maintain the same relative proportion among the synthesized nucleic acid as among the different mRNA species in the sample.

The basic method starts with annealing a poly T oligo with T7 promoter to the poly A end which characterizes all mRNA, synthesizing a single cDNA copy of a particular mRNA, and making multiple RNA copies from the cDNA (FIG.1). Because the cDNA are produced in a one-to-one relationship to the mRNA present in the sample, and the cDNA are copied into RNA multiple times, linear amplification occurs. The nucleic acids are produced in numbers which bear a linear relationship to the mRNA numbers in the sample. The process simultaneously produces copies of many species of mRNA, in proportion to the number of each mRNA species in the sample. The collection of so-produced nucleic acids thus have the same relative representation as the mRNA in the sample.

The reason that this representative collection of nucleic acids is so valuable in the industry is that the collection of mRNA present in a cell indicates cell functions in a in the cell. Numerous different cell samples–from normal to abnormal, from different sites within an organ, and from fetal to aged–have been used to produce distinctive multi-gene profiles which reflect the expression frequencies of a host of genes, providing a detailed snapshot of the sample.

In contrast, the polymerase chain reaction (PCR) and most of the cited prior art have been used to amplify a single species of nucleic acid sequence and its complement, including mRNA in certain circumstances. PCR typically comprises treating separate complementary strands of a

single species of target nucleic acid with two oligonucleotide primers to form complementary primer extension products on both strands that act as templates for synthesizing copies of the desired nucleic acid sequences. By repeating the separation and synthesis steps in an automated system, additional primer extension products are produced and used as the template for additional nucleic acid synthesis. Essentially exponential duplication of the target sequences representing one gene is achieved. The synthesis of highly degenerate or random nucleotide primers (Gould et al., Proc. Natl. Acad. Sci. USA 86: 1934-1938 (1989)) have been implemented to improve the range of cDNAs that can be cloned with PCR; however, the representation of sample nucleic acid is skewed by the number of times that primers anneal to each sample nucleic acid: If more primers anneal to a single nucleic acid species, there are more copies of that gene than if few or one primer anneals to a gene.

In the last Advisory Action, the Examiner rejected claims 42-54 as obvious over Kramer & Lizardi, Patent No. 5,112,734, in view of Hartley Patent No. 5,043,272, and further in view of Miller et al. (Published International Application WO89/06700), Alquist et al. Patent No. 5,466,788 and Kwoh et al.

Applicants believe the rejection of the claims is improper for a number of reasons. First, the Examiner picks and chooses elements from various prior art references that are technically incompatible with each other. This is a classic case of an attempt to reconstruct the invention using hindsight instead of knowledge of one skilled in the art. Second, the Examiner attempts to read claim elements into prior art references which are technically different. The Examiner also ignores language in the claims which clearly narrows the meaning of important terms such as "linearly amplified specific nucleic acid messages" or "relative representation".

The Kramer & Lizardi Patent No. 5,112,734 describes a method for detecting a single species of nucleic acid target sequence (3:43-4) "Transcription of the target-specific gene by a DNA-directed RNA polymerase yields a replicatable RNA which is exponentially amplified by an RNA.directed (sic) RNA polymerase." (3:53-6; emphasis added) Like the PCR method described above, Kramer & Lizardi teach using "two probes [to] serve as primers .. [for] target-specific hybridization..." (3:46-48; emphasis added). The Examiner's assessment of the Kramer disclosure is technically in error.

The Office Action alleges that: 1) this method is a linear method of application; therefore, linear amplification applies; and 2) Kramer recognizes "relative representation".

Because Kramer & Lizardi teach amplification of a single mRNA, it cannot possibly teach the "relative representation" of a collection of nucleic acid species within a multi-gene profile.

The Hartley Patent No. 5,043,272 discloses amplification of sample nucleic acids with random primers. In the art, it is recognized that this method produces copies in direct proportion to the number of times random primers adhere to template nucleic acids – not in proportion to the frequency of the sample nucleic acids. Hartley states that alternatives to random primers may be used, provided that "the degree of amplification may be proportional to the number of priming sites." (5:62-3.)

The Office Action stated that the relative abundance of the amplified mRNAs of Hartley is not necessarily related to the abundance of the mRNAs in the sample.

Applicants agree with this statement and wish to emphasize that this is true because any set of sample nucleic acids will be amplified in direct proportion to the templates' numbers of binding sites for the random primers. According to Hartley, "the degree of amplification may be proportional to the number of priming sites." (5:62-3)

The Miller Patent Publication WO89/06700 provides a method for diagnosing a disease by detecting a single nucleic acid sequence by combining a sample of single-stranded DNA with a promoter-primer. Then the combination is added to a DNA polymerase to produce double stranded DNA. Subsequently an RNA polymerase is added to produce a multiplicity of RNA transcripts of "[t]he nucleic acid to be detected." (12:15) Finally, the presence of the amplified RNA is detected and correlated with the presence or absence of the desired nucleic acid.

The Office Action alleged that this method of amplification uses the RNA polymerase promoter to produce linear amplification and that the method produces amplified RNA transcripts in proportion to the amount of mRNA originally present in the sample.

While it is true that the reference states the proposition that the resulting amount of the single RNA produced is directly proportional to the amount of nucleic acid in the sample, there is no collection of amplified specific nucleic acid messages, so there cannot be a relative representation of specific nucleic acid messages within the sample.

The Alquist & French Patent No. 5,466,788 discloses a new subgenomic promoter (abstract). Its abstract further states that the subgenomic promoter directs the amplified expression of a structural gene in plant tissue by RNA-dependent RNA polymerase. The subgenomic promoter enables synthesis of multiple (-)RNA strands, which in turn are amplified into mRNA for increased protein synthesis. The patent is directed to an improved method of protein synthesis.

Alquist & French state that multiple copies of the subgenomic promoter can direct the synthesis of more than one subgenomic mRNA molecule from one (-) strand RNA template.
The Office Action's assessment of the Alquist reference is incorrect in one important way. The Examiner cited columns 4-5 and 9 and then alleged "the production of ... amplified specific messages" (emphasis added).

In response, Applicants observe that the text at column 4-5 and 9 merely describes "the amplified expression of a structural gene. (*e.g.*, 4:17-19)" Nowhere does the reference describe amplification and production of a collection of multiple species of nucleic acid in a sample. The Kwoh et al. reference (*Proc. Natl. Acad. Sci. USA* 86:1173-7, 1989) is a technique for detecting one amplified HIV sequence in a sample. RNA is transcribed with T7 RNA polymerase promoter into cDNA with RNA polymerase promoter. RNA polymerase is then added to produce many RNA in a process referred to as transcription-based amplification system (TAS), a multi-cycle system. The abstract touts the success of the amplification, which after four cycles is described as an average of "38- to 47-fold per cycle, resulting in a 2-5 x 10^6 fold increase in copy number." It should be noted that 38^4 and 47^4 are within the range of 2-5 x 10^6. Thus, because the copy number increases by the exponent of four, the increase in copy number can be said to be exponential.

The Office Action alleged that Hartley teaches producing linearly amplified specific messages.

Applicants believe this is wrong. In light of the data provided in the abstract and mathematical analysis of the numbers, the paper does not support this conclusion of linear amplification; rather they support the conclusion of exponential amplification.

The Prior Art Does not Disclose or Suggest the Claimed Invention

Whether taken singly or in any combination, the prior art references fail to disclose or suggest the claimed multi-gene profile.

I. The Kramer and Hartley patents do not disclose or suggest all the elements in claims . Neither the Kramer nor the Hartley et al. patents disclose a multi-gene expression profile comprising a collection of linearly amplified specific nucleic acid messages, wherein said amplified specific nucleic acid messages each have an abundance which reflects the relative representation of specific nucleic acid messages within the sample. Hartley teaches the exponential (not linear) amplification of a single nucleic acid species (not a multi-gene

collection). Moreover, because only one gene is amplified, there can be no determination of relative abundance to other nucleic acids in the sample. Hartley teaches amplification with random primers. The nucleic acids in the sample are amplified according to the number of primers annealing to each nucleic acid; thus, the amplified nucleic acid messages in the profile will reflect the number of annealing sites, NOT reflect "the relative abundance" compared to those in the sample.

If the Hartley library (having skewed abundances) were subjected to the method of Kramer, only one sequence from the Hartley library would be amplified. IF somehow, the Kramer technique were altered to enable amplification of the skewed-abundance sequences of Hartley, the sequences would be amplified exponentially and would have no relationship to the relative representation of specific nucleic acid messages within the sample. Combining the teachings of these two references cannot disclose or suggest the invention recited in these claims.

The combination of Kramer and Hartley with Alquist and Kwoh do not disclose all the elements in claims 43-51 53, or 54. Alquist is directed to increased expression of a single structural gene. This reference cannot correct the flaws of the combination of Kramer with the Hartley library that produces a single exponentially amplified sequence or a library of sequences having no relationship to the representation of the nucleic acid messages within the sample.

The combination of Kramer and Hartley with Alquist and Kwoh do not disclose all the elements in claims 43-54. Kwoh discloses exponential amplification of a single nucleic acid sequence. Combining this reference with the combination of Kramer and Hartley adds nothing, because both Kramer and Kwoh are directed to exponential (not linear) amplification. Even if these references are combined with the Hartley library which has genes amplified by the frequency of random primer annealing sites, one cannot achieve the required linearly amplified sequences, nor sequences which reflect the relative abundance of the nucleic acid in the sample.